Vulnerability to cocaine: role of stress hormones

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General discussion

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Individuals may vary widely in susceptibility to acquire cocaine abuse after recreational use of the drug. This individual difference has led to the distinction of phenotypes that are either 'vulnerable' or 'resistant' to the behavioural and reinforcing effects of psychostimulant drugs. Recent studies have identified genes and adverse life events as factors that contribute to psychostimulant sensitivity but how the genetic and environmental inputs (inter)act is still poorly understood.

The objective of this thesis was to investigate the contribution of adrenal stress hormones to individual differences in vulnerability to the psychostimulant effects of cocaine. It was hypothesised that adrenal glucocorticoids contribute to cocaine sensitivity, while their actions are dependent on the *genetic background* of the individual and on the *context* in which these hormones operate. For this purpose, studies were designed to investigate the impact of adrenal hormones on cocaine sensitivity in mice of two genetic backgrounds (C57BL/6 and DBA/2). Only the DBA/2 strain was found to be susceptible to the influence of adrenal hormones on cocaine sensitivity.

The main focus was on the context in which glucocorticoid hormones operate, with emphasis on the timing of the corticosteroid action and the role of the sympathetic nervous system. To that aim, adrenal hormones were surgically and pharmacologically manipulated in the DBA/2 strain by adrenalectomy (surgical removal of the adrenals, 'ADX') and hormone replacement respectively. Behavioural sensitisation, the progressive enhancement of the motor stimulant effects of cocaine with repeated exposure, was used as a read-out parameter as this model reflects long-lasting adaptations in neural circuits involved in motivation and reward. The results show that corticosterone facilitates initiation and retention of the behavioural sensitisation to repeated drug exposure, provided it is continuously circulating. However, in addition to corticosterone, epinephrine is required for full behavioural sensitisation to cocaine in the DBA/2 strain.

In the following paragraphs, the major findings of this thesis are discussed, starting with the importance of genetic background, followed by the critical time-window for the glucocorticoid effects and finally the coordinate actions of corticosterone and epinephrine. Methodological considerations regarding the sensitisation paradigm are discussed in box 1.

1. STRAIN DIFFERENCES IN THE RESPONSE TO COCAINE

*The C57BL/6 and DBA/2 inbred strains represent a model for genetic differences in behavioural responsiveness to cocaine (**chapter 2,** summarised in table 1).* Whereas C57BL/6 mice showed greater locomotor responses to the first cocaine exposure,

Table 1 : *Overview of the locomotor responses of cocaine-treated mice during the sensitisation paradigm.*

Strain	Day 1	Day 9	Increase days 1-9	Day 15*
C57BL/6	↑	↑	no	↑
DBA/2		↑	yes	↑

Arrows indicate a significant difference compared to saline-treated mice of the same strain. No arrow indicates no significant difference. On days 1-9, mice received 15.0 mg/kg cocaine. * On day 15, *all* animals, including the saline-treated control group, received a 7.5 mg/kg cocaine challenge (data from **chapter 2**).

only DBA/2 mice exhibited an increase in drug responsiveness during repeated drug administration. However, both strains expressed sensitisation if challenged with cocaine after a withdrawal interval. The observed strain differences are in line with previous studies showing that C57BL/6 and DBA/2 mice differ in sensitivity to the behavioural, rewarding and reinforcing properties of virtually all classes of abused drugs [43,65,257,447,455]. The present findings are however in disagreement with reports that C57BL/6 mice are less sensitive to the locomotor activating effects of cocaine [483,568,665], whereas one report claimed that neither strain develops behavioural sensitisation during the course of repeated cocaine treatment [483]. Differences in the design of the sensitisation paradigm (box 1) and, in view of the present data, experimental conditions that influence the secretion of adrenal stress hormones, may contribute to variable outcomes across laboratories.

Under the given experimental conditions, three factors are discussed that may underlie the strain differences in behavioural responsiveness to cocaine. These are the pharmacokinetics of cocaine, the properties of the midbrain dopamine system and the stress hormones as modulators of cocaine responsiveness.

First, the psychostimulant may have a different pharmacokinetic profile in the C57BL/6 and DBA/2 strains. However, it has been demonstrated that comparable concentrations of cocaine reach the brain in both strains following i.p. [664,737] or i.v. administration (R. van der Veen, personal communication). This has been contradicted by one report, showing earlier peak concentrations and a longer half-life of cocaine in the brains of C57BL/6 mice after i.p. administration [17]. Given the positive correlation between the rate of psychostimulant bio-availability and the ability to produce behavioural sensitisation [588], the latter finding would imply that cocaine has greater potency to induce sensitisation in the C57BL/6 strain. This is not supported by the present data. However, it could explain the high initial drug response

Box 1: *Behavioural sensitisation: methodological considerations.*

Behavioural sensitisation, the progressive enhancement of the motor stimulant effects of cocaine with repeated exposure, is thought to reflect long-lasting adaptations in neural circuits involved in motivation and reward. Robinson and Berridge first proposed the possible link between behavioural sensitisation and drug addiction. The 'incentive sensitisation' theory implies that drugs of abuse render the neural systems that regulate their motivational aspects hypersensitive, making the drugs increasingly more attractive or 'wanted' [561]. The view that sensitisation is associated with certain aspects of addiction is supported by the observations that: i) sensitisation facilitates subsequent drug taking as measured in self-administration paradigms [295,296,510,607], ii) it reflects adaptations in a common neural substrate, most notably the midbrain dopamine system [708], iii) the neural and behavioural adaptations are very stable and persist until months after the discontinuation of repeated drug treatment [497], iv) the degree of sensitisation may be positively correlated with vulnerability to relapse [152] and, comparable to drug addiction, v) there are considerable individual differences in the propensity to develop behavioural sensitisation [291,559], and vi) sensitisation is influenced by contextual and environmental influences [22,65,510]. However, it is still a matter of debate whether behavioural sensitisation represents initial changes in the addiction process, rather than more advanced stages [36,154,694]. Furthermore, it should be noted that in the present studies, behavioural sensitisation was induced by experimenter-delivered drug administrations. Consequently, this model does not measure voluntary drug seeking or the motivational aspects thereof. It will be essential to extrapolate the present findings to animal models that do, such as the self-administration paradigm. Finally, two aspects of behavioural sensitisation, the time- and context- dependency, require further consideration and are discussed in the following paragraphs.

Behavioural sensitisation is not a unitary phenomenon. First, it represents a time-dependent cascade of cellular and behavioural adaptations [497,520]. The kinetics and direction thereof could be influenced by genetics. Indeed, despite considerable differences in locomotor responsiveness during repeated drug administration, both strains expressed sensitisation in response to a cocaine challenge after a withdrawal interval (**chapter 2**). Furthermore, there were distinct but also partially overlapping neural adaptations in the midbrain dopamine system of C57BL/6 and DBA/2 mice with a history of cocaine exposure (**chapter 3**). Thus, genotype controls both the nature and/or the time course of the neural and behavioural adaptations associated with repeated cocaine exposure. Therefore, the observed strain differences in behaviour and neural correlates are representative for the time point at which they were measured, whereas further research is required to investigate their significance after e.g. longer episodes of withdrawal.

Second, at least two types of behavioural sensitisation can be distinguished: context-independent and -dependent sensitisation. Whereas the former relies primarily on the pharmacological actions of the drug, the latter involves learned associations between the psychostimulant effect of the drug and otherwise neutral stimuli. Both phenomena may co-exist, however the extent to which they contribute to the behavioural hyperresponsiveness can be influenced by genetics. Indeed, the C57BL/6 strain is more sensitive to context-dependent sensitisation [20,65,559] and exhibits greater spatial and contextual memory [10,499,581,677], when compared to the DBA/2 strain. In the present studies, animals received cocaine both in the test cage and in the home cage on several occasions, a setting which may promote the development of either type of sensitisation. An important challenge is to unravel which neuronal substrates contribute to context dependent vs. -independent sensitisation.

of C57BL/6 mice [729], which may have masked subsequent behavioural sensitisation (**chapter 2**).

Second, the strain difference in the midbrain dopamine system that mediates the behavioural and reinforcing effects of psychostimulant drugs, may play a role in the differential cocaine responsiveness (**chapter 3**, [69,199,470,737]). The few studies that have investigated neurotransmitter systems other than dopamine, point to strain differences in GABA-ergic-, glutamatergic- and endogenous opioid-signalling [114,156,157,313,453,471,718]. However, their contribution to the strain difference in cocaine responsiveness remains to be established and would be an interesting line of further research. In section 1.1 further aspects of the role of dopamine are discussed.

Third, the adrenal glucocorticoids may modulate behavioural responsiveness to cocaine. This argument is based on the wealth of data showing that the glucocorticoids contribute to psychostimulant sensitivity [168,240,418,421,424,516], the notion that psychostimulant drugs activate the HPA-axis [31,281,355,448,461,590], and the observed strain differences in HPA-axis function [66,321,622]. Therefore, an essential aspect of the strain comparison in this thesis was to characterise HPA-axis responsiveness to cocaine and to investigate the role of adrenal hormones in behavioural sensitisation (see sections 1.2 and 1.3, respectively).

1.1 Dopamine

*The two strains are characterised by pronounced differences in the mesocorticolimbic and nigrostriatal dopamine systems (**chapter 3**, see table 2).* The most direct measure of dopaminergic transmission is dopamine release. Compared to DBA/2 mice, C57BL/6 mice display greater amphetamine-induced dopamine release in the NAc [700,760], which is a critical determinant of the behavioural response to the drug [700]. The observed differences in initial cocaine responsiveness (**chapter 2**), suggest that a comparable phenomenon applies to cocaine. This appears to be contradicted by the higher TH and DAT mRNA expression in cell body regions of the DBA/2 strain (**chapter 3**), which is suggestive of a higher tone in the DBA/2 dopamine system. However, the increased expression of all presynaptic markers appeared to be fully attributable to the higher number of dopaminergic (TH^+) neurons in this strain. In fact, the latter observation, together with the notion that basal dopamine output is comparable in the two strains [760], suggests that there must be greater inhibition of dopaminergic transmission in the DBA/2 strain. The PFC may play an important role therein, as dopamine in the mPFC controls subcortical dopamine release in a strain-dependent manner [700,701]. Further studies applying microdialysis to measure dopamine release and turnover in response to cocaine in the NAc and PFC are required to investigate this issue.

Table 2 : *Overview of the strain differences in the basal dopamine system of DBA/2 relative to C57BL/6 mice.*

		DBA/2 vs. C57BL/6
TH	**SNc**	↑
	VTA	↑
DAT	**SNc**	↑
	VTA	
D2	**SNc**	↑
	VTA	↑
TH$^+$ cells	**SNc**	↑
	VTA	↑
D1	**NAc core**	↑
	NAc shell	↑
	rCP	
	cCP Lat	
	cCP DM	
	cCP VM	
D2	**NAc core**	↓
	NAc shell	
	rCP	↓
	cCP Lat	↑
	cCP DM	
	cCP VM	

Arrows indicate a significant difference compared to C57BL/6 mice. No arrow indicates that there is no significant difference. TH: tyrosine hydroxylase, DAT: dopamine transporter, SNc: substantia nigra pars compacta, VTA: ventral tegmental area, NAc: nucleus accumbens, (r/c) CP (Lat,DM,VM): (rostral/caudal) caudate putamen (lateral, dorsomedial, ventromedial regions) (data from **chapter 3**).

Whereas several studies have demonstrated strain differences in dopamine receptor densities or binding, their role in behavioural responsiveness to psychostimulants remains to be established. A consistent finding across laboratories is the strain difference in D2 receptor density in distinct terminal fields (higher in C57BL/6) and the cell body regions (higher in DBA/2) of the mesocorticolimbic and nigrostriatal dopamine system (**chapter 3**, [69,199,470]). The latter finding has led to the hypothesis

that DBA/2 mice have greater auto-inhibition of dopaminergic transmission [69,531]. However, in view of the abovementioned strain difference in neuron number, it remains to be investigated how much of the lower D2 binding is attributable to the fewer number of dopaminergic neurons in this strain. The higher D2 binding in the CP of C57BL/6 mice could be a marker of greater cocaine responsiveness, given that apomorphine-susceptible rats (APO/SUS, see section 2), that are more responsive to psychostimulant-induced locomotion than APO/UNSUS rats [127], also display greater D2 binding in the CP when compared to their counterparts [576]. However, in the C57BL/6 and DBA/2 mouse strains this difference is subregion-dependent, as DBA/2 mice displayed greater D2 binding in the lateral subdivision of the caudal CP. With respect to D1 receptor binding in the terminal fields, conflicting results have been obtained and this is an issue that requires further investigation (**chapter 3**, [69,199,470]). One approach to investigate the functionality of the strain differences in D1 and D2 receptor density in the different subfields could involve local administration of receptor agonists and antagonists. Furthermore, there are now ligands with relative selectivity for the 5 individual dopamine receptor subtypes, the contribution of which could be another interesting line for further research.

In the present studies, strain differences in behavioural responsiveness to cocaine were related to 'candidate genes' in the midbrain dopamine system. It should be noted that these markers do not prove a causal relationship and further research is required to evaluate their role in cocaine responsiveness. In addition, it would be interesting to search for novel genes, e.g. by microarray screening. This technique may allow the identification of novel pathways that underlie the strain differences in cocaine responsiveness. Furthermore, recombinant inbred (RI) strains (crossings between C57BL/6 and DBA/2 strains) can be used to determine quantitative trait loci (QTL), sites on a chromosome that contain genes that influence a given behaviour. This approach has already been applied for cocaine-induced seizures [273], acute and sensitised locomotion [507,664]. Interestingly, one study found a QTL in the region of chromosome 9 that contains the drd2 gene which encodes the D2 receptor [273]. Finally, there are two different splice variants of the D2 receptor ('short' and 'long') [107,139,238,463] and evidence is emerging that there are polymorphisms and single-nucleotide-polymorphisms (SNPs) in the dopamine receptors. Especially the D4 receptor is unique in having an exceptionally high number of polymorphic sites [96,477,651,690], some of which have been associated with psychosis [96], novelty seeking personality traits [37,188] and heroin abuse [652] in humans. It would be interesting to investigate whether there are such polymorphisms in the D2 and D4 receptors in the C57BL/6 and DBA/2 strains.

In conclusion, there are profound differences in the midbrain dopamine system of C57BL/6 and DBA/2 mice that may underlie the strain differences in behavioural responsiveness to psychostimulants. However, the nature of the crucial event(s) in dopamine signalling requires further investigation.

1.2 HPA-axis

The C57BL/6 and DBA/2 strains displayed very different endocrine responses to cocaine. In contrast to the HPA-axis activation and endocrine sensitisation in DBA/2 mice, cocaine attenuated HPA-axis reactivity in C57BL/6 mice throughout the sensitisation paradigm (**chapter 2,** summarised in table 3).

The endocrine sensitisation observed with repeated cocaine exposure in the DBA/2 strain is in agreement with most earlier observations in rats [29,51,387,610,694]. The data from **chapters 2** and **5** suggest that sensitisation takes place at the level of the PVN and may in addition involve adrenal hyperresponsiveness to ACTH. In support of this, HPA-axis sensitisation to amphetamine is associated with neuroplastic changes in the PVN [315]. Also, chronic cocaine treatment increases the sensitivity of adrenocortical cells to the stimulatory effects of ACTH on steroidogenesis [369]. Furthermore, the data in **chapter 5** point to a role for the adrenal epinephrine in HPA-axis sensitisation at the level of the PVN. Since epinephrine itself cannot cross the blood-brain-barrier, the catecholamine most likely influences brain function via β-adrenoreceptors on vagal afferents to the CNS. The contribution of epinephrine to HPA-axis sensitisation could be tested by administration of a peripheral β-blocker prior to cocaine administration.

The attenuation of HPA-axis activation by cocaine in the C57BL/6 strain was unexpected and this phenomenon has, to our knowledge, not been reported previously. In line with the present findings, C57BL/6 mice display lower, though not

Table 3 : *Overview of corticosterone secretion of cocaine-treated mice during the sensitisation paradigm.*

Strain	Day 1	Day 9	Increase days 1-9	Day 15*
C57BL/6	↓	(trend ↓)	no	
DBA/2	↑	↑↑	yes	↑

Arrows indicate a significant difference compared to saline-treated mice of the same strain. No arrow indicates no significant difference. On days 1-9, mice received 15.0 mg/kg cocaine. * On day 15, *all* animals, including the saline-treated control group, received a 7.5 mg/kg cocaine challenge (data from **chapter 2**).

reduced, corticosterone secretion in response to ethanol when compared to DBA/2 mice [553]. Opposite strain differences exist for endocrine responsiveness to mild stressors, such as novelty (S. Dalm, personal communication, [66]) or saline administration in the test setting (**chapter 2**), in response to which C57BL/6 mice show a greater increase in corticosterone secretion than DBA/2 mice. By contrast, more severe stressors (electrical shock, restraint) induce greater HPA-axis activation in the DBA/2 strain [68,321,622]. Therefore, the degree of endocrine responsiveness to cocaine and various stressors is not necessarily correlated. Depending on their nature and severity, these 'stimuli' appear to recruit different neuronal pathways that regulate activity of the HPA-axis.

Several mechanisms could contribute to the differential HPA-axis responsiveness of C57BL/6 and DBA/2 mice to cocaine.

First, pre-existing differences or drug-induced alterations in functioning or wiring of the HPA-axis may play a role. Cabib *et al.* demonstrated higher GR and MR binding capacity *in vitro* in hippocampal cytosol of DBA/2 mice [68]. Whether this plays a role in the strain differences in HPA-axis activity remains to be established, and additional investigation of HPA-axis parameters at the level of the PVN and pituitary is required. Furthermore, given the strain-difference in endocrine adaptation to repeated cocaine exposure, it would be interesting to investigate neural and functional aspects of the HPA-axis in sensitised mice. In humans, there is limited evidence that drugs of abuse influence negative feedback regulation of the HPA-axis [14,174]. Could it be that cocaine alters glucocorticoid negative feedback in a strain-dependent manner? This question could be addressed by performing a dexamethasone suppression test in addition to measuring GR densities in the pituitary and PVN.

We found that DBA/2 mice have considerably higher AVP mRNA expression (on average 25%) in the magnocellular and parvocellular neurons of the PVN (data not shown). AVP expression and release are under monoaminergic control [381,745], suggesting that they can be regulated by cocaine. Interestingly, AVP may not only play a role in endocrine-, but also in behavioural responsiveness to cocaine. There is considerable evidence that AVP reduces sensitivity to the behavioural (locomotion, sensitisation) and reinforcing effects of drugs of abuse [108,155,362,600,687]. Recently, it was demonstrated that environmental manipulations that increase cocaine self-administration in the DBA/2, but not the C57BL/6 strain, are associated with a decrease in AVP mRNA expression in the extended amygdala of the DBA/2 strain only (R. van der Veen *et al.*, personal communication). This suggests that extrahypothalamic AVP engages in gene x environment interactions that correlate with sensitivity to the reinforcing effects of cocaine. The role of hypothalamic

and extrahypothalamic AVP in the observed strain differences in endocrine and behavioural responsiveness to cocaine could be further investigated by e.g. targeted knock-down or overexpression of AVP.

Second, there could be strain differences in the neurotransmitter systems that regulate HPA-axis activity. Given i) the monoaminergic actions of cocaine, ii) the strain differences in the dopamine system (**chapter 3**), and iii) the role of dopamine and norepinephrine in HPA-axis activation [52,113,226,382,398], it is tempting to hypothesise a role for the catecholaminergic innervation to the PVN. Indeed, as proposed in **chapter 5**, norepinephrine may play a role in HPA-axis sensitisation in DBA/2 mice. Further characterisation of the monoaminergic innervation of the PVN in the two strains is required to address this issue.

Third, the differences in HPA-axis responsiveness to cocaine may reflect strain differences in coping with drug-induced arousal [454] or in the perception of stressful events (e.g. test- and treatment procedures). In these cases, higher brain centers involved in perception, appraisal, cognitive processes and emotional arousal exert indirect control over HPA-axis activity.

In view of the working hypothesis that adrenal glucocorticoids contribute to the strain differences in behavioural responsiveness to cocaine, it is of interest to compare locomotor- and corticosterone responses to the psychostimulant. In the C57BL/6 strain, there was a clear dissociation between the effect of cocaine on locomotion (increased) and corticosterone secretion (attenuated) on all test days. Furthermore, C57BL/6 mice displayed behavioural, but not endocrine, sensitisation in response to the cocaine challenge. In the DBA/2 strain, by contrast, cocaine stimulated locomotion and corticosterone secretion, and both responses sensitised with repeated drug exposure.

In summary, the C57BL/6 and DBA/2 inbred strains differ not only in behavioural, but also in HPA-axis responsiveness to cocaine. Furthermore, there was a striking parallel between behavioural and endocrine sensitisation in the DBA/2 strain. This raises the question whether the HPA-axis is involved in behavioural sensitisation, and whether it contributes to the differences in cocaine responsiveness between the strains.

1.3 Role of adrenal hormones

*The DBA/2, but not the C57BL/6 strain, is susceptible to the impact of adrenal hormones on behavioural sensitisation to cocaine (**chapter 2**).* ADX prevented initiation and retention of behavioural sensitisation in the DBA/2 strain only. However, the

strain differences in behavioural responsiveness to cocaine were not diminished, but rather enhanced by ADX. By contrast, a period of food shortage abolished the strain differences in amphetamine-induced place preference and locomotion by increasing responsiveness selectively in the DBA/2 strain [71]. This is likely to depend on stress-induced corticosterone secretion, since it could be blocked by a glucocorticoid antagonist [420]. These data point to an important facilitatory role for corticosteroid hormones in behavioural responsiveness to psychostimulants in the DBA/2, but not the C57BL/6 strain.

Is it a general phenomenon that the C57BL/6 strain is relatively resistant to glucocorticoids, or does this depend on the experimental design or the read-out parameter? Among experimental parameters that could influence susceptibility to adrenal hormones are the dosage of the psychostimulant and the design of the sensitisation paradigm, such as e.g. the context in which the drug was administered. The dose of 15.0 mg/kg cocaine resulted in pronounced locomotor activation in the C57BL/6, but not the DBA/2 strain (**chapter 2**). It is conceivable that for C57BL/6 mice, this dosage was high enough to override the influence of other factors, including adrenal hormones. In this respect, it would be interesting to establish a dose-response curve for cocaine-induced behavioural sensitisation in SHAM and ADX mice.

Alternatively, different types of behavioural tasks might reveal sensitivity to adrenal hormones in the C57BL/6 strain. If compared to DBA/2 mice, C57BL/6 mice exhibit greater spatial and contextual memory [10,499,581,677], which has been related to strain differences in hippocampal function [575]. In support of this, cocaine-treated C57BL/6 mice displayed greater locomotor responses when exposed to the test context in the absence of cocaine (**chapter 2**). The C57BL/6 strain could be susceptible to the influence of adrenal hormones in a sensitisation paradigm designed to exclusively reveal context-dependent sensitisation (see also box 1), or in behavioural tasks that strongly depend on hippocampal function (e.g. radial arm maze, fear conditioning, Morris water maze). Indeed, in C57BL/6 mice, exogenous corticosterone and stress modulate spatial learning in water maze and circular hole board tasks [265,266] and memory consolidation in tests for passive avoidance [68,95]. Therefore, the relative resistance of C57BL/6 mice to adrenal hormones or environmental manipulations is not a general phenomenon for this strain, although it has been consistently reported for behavioural responsiveness to psychostimulant drugs. This could indicate that neural mechanisms that mediate psychostimulant responsiveness are relatively resistant to glucocorticoid hormones in the C57BL/6 strain. *Indeed, the DBA/2, but not the C57BL/6 strain, was susceptible to the impact of adrenal hormones on neuroadaptations associated with repeated cocaine exposure (**chapter 3,** summarised in table 4).*

Table 4: *Overview of the adaptations in the dopamine system of mice subjected to the sensitisation regimen.*

		DBA/2		C57BL/6	
		SHAM	**ADX**	**SHAM**	**ADX**
TH	**SNc**	↑			
DAT	**SNc**	↑			
D2	**NAc core**		↓*		
	rCP		↓*		
	cCP DM	↓		↓	

SHAM operated and adrenalectomised (ADX) mice were subjected to the sensitisation regimen and sacrificed 24 hours after the cocaine challenge under basal conditions. Arrows indicate a significant difference in cocaine- compared to saline-treated mice of similar surgery and strain. No arrow indicates that there is no significant difference. * Significantly different from cocaine-treated SHAM mice. TH: tyrosine hydroxylase, DAT: dopamine transporter, SNc: substantia nigra pars compacta, NAc: nucleus accumbens, (r/c) CP (DM): (rostral/caudal) caudate putamen (dorsomedial region) (data from **chapter 3**).

The data point to a role for the nigrostriatal dopamine system and the NAc core in mediating the effects of adrenal hormones in a strain dependent manner. In the DBA/2 strain, sensitised SHAM mice displayed increased expression of presynaptic markers, whereas sensitisation-resistant ADX mice were characterised by reduced D2 binding in the terminal fields of the NAc core and rostral CP. These changes were not observed in C57BL/6 mice that developed sensitisation independent of the adrenals. Although speculative, these data point to i) greater reactivity of the nigrostriatal neurons in SHAM mice, and ii) reduced potency for postsynaptic signalling via the D2-like receptors in ADX mice, of the DBA/2 strain. Further research is required to test these hypotheses, and to determine which of the changes are critical for the behavioural response to cocaine and the influence of adrenal hormones. One approach could involve overexpression of TH and DAT in ADX mice, or conversely, knock down of D2 receptors in SHAM mice. Furthermore, it would be of interest to investigate these markers after longer episodes of withdrawal, given that neural adaptations associated with behavioural sensitisation are characterised by marked time-dependency [497,520]. In addition, it will be necessary to investigate the contribution of the D2 and D3 receptors individually, in view of their presumed opposite effects on psychostimulant-induced locomotion.

With respect to the D2 receptor, there is considerable evidence that D2-like dopamine receptors engage in gene x environment interactions. In humans, variations in D2 receptor alleles have been associated with drug addiction [121,473,475,476]

and stress differentially affects cigarette craving or performance in a cognitive task between carriers and non-carriers of the A1 allele [41,198]. Furthermore, stress induces opposite alterations in D2 receptor binding in the C57BL/6 and DBA/2 strains [69]. In view of the affinity of the D2-like receptor ligand iodosulpride for both D2 and D3 receptors, it is interesting to note that the D3 receptor has been suggested to play a role in gene x environment interactions in schizophrenia and other psychotic disorders [490]. In addition, studies in laboratory rodents have demonstrated that the D3 receptor contributes to stress-induced reinstatement of cocaine seeking [740]. Thus, signalling via D2-like receptors may be subject to regulation by adrenal hormones in a genotype-dependent manner.

Finally, the lack of major changes in the dopamine system of sensitised C57BL/6 mice suggests that other neurotransmitter systems play a more prominent role in sensitisation of this strain. A likely candidate is glutamate, given that the C57BL/6 strain is relatively more susceptible to contextual information, and glutamatergic transmission has been proposed to play a prominent role in context-dependent sensitisation [10,35,499,581,677]. In addition, glutamate may contribute to the effects of glucocorticoids on behavioural sensitisation in the DBA/2 strain. There is considerable evidence that adrenal glucocorticoids modulate glutamatergic transmission in the limbic system, e.g. in the midbrain dopamine system, hippocampus and prefrontal cortex [2,275,400,414,460,638,720]. These effects of corticosterone have been most extensively characterised for the hippocampus and involve regulation of glutamate receptor expression [275,414], glutamate release [400,460,638] and transmission via NMDA and AMPA receptors [337,655]. It has been demonstrated both *in vitro* and *in vivo* that corticosterone potentiates responses of VTA dopaminergic neurons to excitatory amino acids, although there is controversy regarding the roles of GR and MR [110,488]. The time course of the effects of corticosterone in the study of Cho *et al.* suggests that this may involve non-genomic actions of the hormone [110], which is in good agreement with a growing body of evidence that glucocorticoids can modulate excitatory transmission via a rapid, non-genomic mode of action involving membrane-bound receptors [175,338,466,655,699,759]. By contrast, other effects of glucocorticoids on glutamatergic transmission require genomic mechanisms [337]. Taken together, the direct facilitatory effect of glucocorticoids on responsiveness of midbrain dopamine neurons to excitatory amino acids, in combination with the actions on glutamatergic transmission in other limbic regions such as the hippocampus, may facilitate responsiveness to psychostimulant drugs. It would be of great interest to further investigate the interplay between glucocorticoids and glutamate in behavioural sensitisation to cocaine with special emphasis on genomic- vs. non-genomic mechanisms. Especially given the present observation that a modest degree of sensitisation was observed in DBA/2 mice receiving corticosterone 5 min. prior to

cocaine administration (**chapter 4**, see section 3.2 for discussion). In addition, two recent studies have demonstrated that the rapid effects of glucocorticoids on glutamate release in the hypothalamus are mediated via endocannabinoid release and subsequent CB1 receptor activation [175,176]. Given the role of endocannabinoids in drug addiction, it would be interesting to investigate whether a similar mechanism applies to the contribution of corticosterone to behavioural sensitisation. Finally, in view of the present data, an important line of further research involves comparing the C57BL/6 and DBA/2 mouse strains with respect to the interaction between corticosteroids and glutamate in behavioural sensitisation to psychostimulants.

*In summary, the evidence presented in **chapters 2** and **3** shows that the two inbred strains provide a model for genetic differences in neural, behavioural and endocrine responsiveness to cocaine. Furthermore the DBA/2, but not the resistant C57BL/6 strain, is susceptible to the impact of adrenal stress hormones on cocaine sensitivity at the level of both dopaminergic neurotransmission and behavioural responsiveness.*

2. OTHER MODELS FOR INDIVIDUAL DIFFERENCES IN PSYCHOSTIMULANT SENSITIVITY

Individual differences in psychostimulant sensitivity have gained increasing attention during the past decades. A number of models have been developed in which animals were selected based on genetics (inbred rat or mouse strains) [214,485,626], or pre-existing traits [44,203,237,287,290,354,378,509,684]. These models may reflect different motivational aspects of drug-taking that have also been distinguished in humans [268]: to 'seek novelty (sensation)' [509] or to 'alleviate negative affect' [287]. In the following paragraphs, the C57BL/6 and DBA/2 strains are compared to other selected animals such as e.g. the so-called high- (HR) and low responder (LR) rats of Piazza *et al.*, to which they bear striking resemblance. It should be noted that inbred strains represent one particular set of genes and it is fraught with difficulty to draw a direct parallel to outbred strains.

The HR and LR rats are distinguished from an *outbred* population, based on behavioural reactivity in a novel environment (above or below the mean, respectively) [509]. These sub-populations of animals have certain phenotypic characteristics that have been observed in various animal models. A positive correlation may exist between behavioural reactivity in a novel environment [200,232,511] and i) the locomotor response to acute administration of psychostimulants [82,203,290,378,509], ii) psychostimulant-induced dopamine release in the NAc [82,236,290,378,578] and iii) the propensity to self-administer psychostimulants [232,354,509]. In addition, these parameters have

been positively correlated with the endocrine response to stress, although this is an issue of debate [255,511,716]. These characteristics are however not consistent across all models [287,684]. The APO/SUS and -UNSUS rat lines, bred from rats that were selected for apomorphine-induced gnawing (gnawing score: APO/SUS: >500/45 min, -UNSUS: <10/45 min in response to 1.5 mg/kg apomorphine) are an example. Whereas APO/SUS rats are more responsive to novelty and psychostimulant-induced locomotion, they self-administer less cocaine under habituated conditions [126,127,684]. However, these animals were selected and bred for a dopaminergic phenotype, which makes them essentially different from the HR/LR rats.

The notion that a cluster of phenotypic parameters is observed across laboratories in animals with different genetic backgrounds, indicates that these are representative for at least a sub-population of individuals. The C57BL/6 and DBA/2 strains display differences in behavioural and endocrine responsiveness to novelty comparable to those of the HR and LR rats, respectively (personal observations, [66]). The HR rats show greater amphetamine-induced locomotion when compared to LR rats, and these differences are abolished with repeated drug exposure by a progressive increase in the LR, but not the HR group [509], although this may depend on experimental parameters [179,291]. Similarly, whereas C57BL/6 mice were more responsive to the first cocaine exposure, only DBA/2 showed an increase in drug responsiveness during the treatment period (**chapter 2)**. Furthermore, C57BL/6 mice and HR rats show greater psychostimulant-induced dopamine release in the NAc and propensity for amphetamine self-administration, when compared to DBA/2 mice and LR rats respectively [290,354,447,509,701,760].

The parallel between the HR/LR rats and the two mouse strains also extends to susceptibility to the effects of corticosterone and stressors. Administration of corticosterone (prior to a self-administration session or in the amphetamine solution) and social defeat stress facilitate psychostimulant self-administration in LR rats, while decreasing it in HR rats [323,516]. Similarly, adrenal hormones (**chapter 2**) and environmental manipulations [20,71] facilitate behavioural responsiveness to cocaine and amphetamine in the DBA/2, but not the C57BL/6 strain. A comparable phenomenon has been observed for the Fischer 344 and Lewis inbred rat strains. Fischer 344 rats (comparable to LR rats in terms of behavioural responsiveness to novelty and psychostimulants), were susceptible to the influence of corticosterone on cocaine sensitisation, whereas Lewis rats (HR-like) were not [485]. However, with respect to novelty-induced corticosterone secretion, these rat strains are distinct from the HR/LR rats, with Fischer 344 rats having higher corticosterone secretion than Lewis rats [255]. There are also differences between the HR/LR rats and the two inbred mouse strains. Whereas exposure to similar concentrations of corticosterone abolishes the difference in amphetamine-induced locomotion between HR and LR rats [516], the C57BL/6

Table 5: *Overview of parameters of the dopamine system in various animal models for individual differences in sensitivity to psychostimulants.*

		HR/LR	C57/DBA	SUS/UNSUS	LEW/FIS	Reference
D2	**NAc**	↓ (p)	↑ (p)	↔ (p)	↓ (p)	69,214,292,576*
	CP	↓ (p)	↔ ↓ ↑ (p)	↑ (p)	↓ (p)	69,199,214,292,470,576*
	VTA		↓ (p)			69*
	SNc		↓ (p)	↔ (p)		69,576*
D1	**NAc**	↑ (p)	↔ ↓ (p)	↔ (p)	↔ (p)	69,214,292,576*
	CP	↔ (p)	↔ ↑ (p)	↔ (p)	↔ (p)	69,199,214,292,470,576*
TH	**NAc**	↔ (p)		↑ (p)	↓ (p)	32,408,682
	CP	↔ (p)			↔ (p)	32,408
	VTA	↔ (m) ↓ (p)	↓ (m)	↔ (m)	↑ (p)	32,293,408,576 *
	SNc	↔ (m) ↔ (p)	↓ (m)	↑ (m)	↔ (p)	32,293,408,576*
DAT	**NAc**	↔ (p)			↓ (p)	214,292
	CP	↔ (p)			↓ (p)	214,292
	VTA		↓ (m)			*
	SNc		↓ (m)			*

Arrows indicate significant difference compared to LR, DBA/2, APO/UNSUS or Fischer 344. p: protein, results obtained with radioligand binding or immunostaining, m: mRNA, measured by *in situ* hybridisation, TH: tyrosine hydroxylase, DAT: dopamine transporter, NAc: nucleus accumbens, CP: caudate putamen, VTA: ventral tegmental area, SNc: substantia nigra pars compacta, * data from the present studies. It should be noted that the binding studies are not fully comparable due to the use of different ligands with different affinities for the dopamine receptor subtypes.

and DBA/2 strains by nature show opposite strain differences in cocaine-induced locomotion and corticosterone secretion (**chapter 2**). Therefore, it is not possible to draw a general conclusion regarding the relationship between the HPA-axis and psychostimulant responsiveness across models. Further analysis of corticosterone concentrations *prior* to drug administration and, given the present data, *in response to* the psychostimulant, would be an interesting line of further research.

At the level of dopaminergic transmission, there appears to be little consistency between the models (see table 5). For example, the dopaminergic markers in drug naïve animals of the different 'high-low' responder pairs (HR-LR, C57BL/6-DBA/2, Lewis-Fischer 344) do not show a consistent pattern. Furthermore, stress- or corticosterone-induced dopamine release in the NAc is higher in HR rats, but lower in C57BL/6 mice [73,518,580,683,703], when compared to their counterparts. In addition,

whereas the DBA/2 strain is most susceptible to the impact of ADX on drug-induced adaptations in the dopamine system (**chapter 3**), ADX selectively reduces stress-induced dopamine release in the HR rat [580]. This indicates that, except for dopamine release in response to psychostimulants, other markers of the dopamine system do not seem to be predictive for psychostimulant-induced behaviour *across* models. This does, however, not exclude that in any particular setting or strain dopamine is the key determinant for psychostimulant sensitivity.

In summary, the C57BL/6 and DBA/2 mice show resemblance to animals that are selected on the basis of high and low 'novelty seeking'. Interestingly, it appears that stressors or exogenous corticosterone facilitate behavioural responsiveness to psychostimulants in less sensitive individuals, such as the LR rats, Fischer 344 rats and DBA/2 mice. By contrast, 'high-responders' appear less susceptible to the influence of corticosterone. This interpretation should be regarded with caution, given that the 'HR/LR' phenotype in psychostimulant responsiveness might depend on the dose of the psychostimulant (see section 1.3), as well as on the experimental design, a good example being the susceptibility to context dependent (higher in C57BL/6) versus -independent (higher in DBA/2) sensitisation of the two inbred strains (see box 1) [65,559]. Furthermore, it would be interesting to characterise the C57BL/6 and DBA/2 strains for behavioural parameters that reflect coping with negative affect, e.g. stress-induced self-grooming, which has been demonstrated to predict cocaine self-administration [287].

In summary, the genetic differences in behavioural responsiveness to novelty and psychostimulant drugs are not unique to the C57BL/6 and DBA/2 strains. The HPA-axis plays a prominent role in psychostimulant responsiveness, which is especially evident in animals that are less sensitive to the drugs under the given experimental conditions. However the exact contribution of adrenal hormones may vary with the genetic background, which is consistent with the view that there are considerable individual differences in susceptibility to the impact of glucocorticoids on psychostimulant sensitivity.

3. THE CONTEXT OF STRESS HORMONE ACTION

The actions of adrenal glucocorticoid hormones are highly context-dependent [318]. However, despite convincing evidence that glucocorticoids mediate the effects of stress on psychostimulant sensitivity, the context- and time-dependency of their actions is poorly understood. The studies presented in **chapters 4** and **5** were therefore designed to investigate the role of adrenal glucocorticoids in behavioural

sensitisation to cocaine in relation to i) the stage of behavioural sensitisation, ii) the time of drug exposure, and iii) the activity of the sympathetic nervous system. The DBA/2 strain was used because of its susceptibility to the impact of adrenal hormones.

3.1 Stage of behavioural sensitisation

Glucocorticoids play a role during the initiation, rather than the expression of cocaine sensitisation in DBA/2 mice. Administration of the GR antagonist mifepristone to previously sensitised animals failed to block expression of locomotor sensitisation to cocaine (**chapter 4**), whereas substitution of ADX mice from the start of the sensitisation paradigm with chronic corticosterone alone (**chapter 4**), or in combination with epinephrine (**chapter 5**), respectively partially and fully restored the deficit in behavioural sensitisation.

The role of glucocorticoids in initiation of sensitisation is supported by previous studies [498,527] and points to a coordinate mechanism of action of glucocorticoids and psychostimulant drugs in facilitating neurochemical and behavioural adaptations. In support of this, glucocorticoids can increase extracellular concentrations of dopamine by i) increasing the levels of TH [485,637], ii) decreasing dopamine catabolism [397], and iii) decreasing catecholamine re-uptake [598], but see also [186,397]. Furthermore, glucocorticoids may control dopaminergic transmission through other neurotransmitter systems that modulate the activity of DA neurons [459,488]. Stressors and exogenous glucocorticoids increase psychostimulant-induced dopamine release in the NAc [77,164,518,577], induce (cross-) sensitisation to the neurochemical-, behavioural- and reinforcing effects of psychostimulants [165,166,472,493,510], and facilitate psychostimulant self-administration already during the acquisition phase [244,418,516]. Furthermore, adrenal glucocorticoids can induce synaptic alterations on dopaminergic neurons similar to those induced by drugs of abuse [582] and are readily self-administered by laboratory rodents [512]. Finally, in humans, high glucocorticoid concentrations can induce 'steroid psychoses' [274] and adverse life experiences may precipitate drug use and abuse.

Whereas there is a considerable body of evidence that glucocorticoids contribute to initiation and acquisition of hyperresponsiveness to the behavioural and reinforcing effects of psychostimulants (in animals with certain genetic backgrounds), controversy exists regarding the contribution of corticosteroids to sustained expression of these behaviours once they have been established. Indeed, the present data (**chapter 4**) do not support a role for glucocorticoids in expression of behavioural sensitisation to cocaine, which is in agreement with observations that ADX performed in already sensitised animals does not prevent expression of the behavioural

hyperresponsiveness [527,529]. However, other studies have shown that mifepristone can reduce the motivation to self-administer cocaine, and block amphetamine-induced behavioural sensitisation, in rats that had previously acquired these behaviours [153,168]. Furthermore, stress and glucocorticoids can play a role during maintenance [92,131,245,246,417] and reinstatement [163,194,517] of psychostimulant self-administration. Therefore, it appears that glucocorticoids can contribute to expression of these behaviours, whereas the experimental parameters and possibly genetic influences determine whether this is revealed. Indeed, Deroche-Gamonet *et al.* demonstrated that mifepristone only reduces motivation for cocaine self-administration in a subset of outbred rats that display an exceptionally high drug response [168]. Furthermore, the length of the withdrawal duration and the extent to which behavioural sensitisation has developed, may determine whether its expression is susceptible to adrenal hormones or environmental manipulations at a given time point.

In conclusion, adrenal glucocorticoids contribute to initiation of cocaine-induced behavioural sensitisation in DBA/2 mice. A next step towards understanding the context in which glucocorticoids operate, was to investigate the time-window for the actions of corticosterone within the initiation phase. To achieve this, ADX mice were given corticosterone replacement either 5 min. or 2 hrs. prior to each drug exposure, or continuously via release from a s.c. implanted pellet.

3.2 Time dependence: mode of corticosterone replacement

*Neither of the three corticosterone replacement regimens was effective in fully restoring behavioural sensitisation in ADX mice (**chapter 4**, see table 6)*. This is intriguing, given the vast body of evidence that glucocorticoid hormones contribute to the neurochemical and behavioural effects of psychostimulants, as outlined in the previous section. In agreement with reports that repeated stress and chronic administration of high doses of corticosterone can enhance locomotor responsiveness and behavioural sensitisation to psychostimulants [77,284,383,485,498], the continuous hormone replacement via the s.c. pellet was effective in (partially) restoring behavioural sensitisation to cocaine in ADX mice. However, the data suggest that the time-kinetics of the sensitisation in mice receiving chronic corticosterone is different from that observed in SHAM-operated animals and may require a longer withdrawal duration (**chapters 4** and **5**).

The continuous and stable corticosterone level achieved through a slow release pellet does not represent a physiological condition, because it does not mimic the ultradian and circadian rhythms in circulating corticosterone concentrations [751]. Therefore, the time-window for the action of glucocorticoids requires further

Table 6: *Overview of the locomotor responses of cocaine-treated DBA/2 mice receiving various regimens of corticosterone replacement during the sensitisation paradigm.*

Method	Day 1	Day 9	Increase days 1-9	Expression day 15*	Day 21
5 mins.			yes (minor)	no	
2 hrs.			no	no	
chronic	↓	↑	yes	no	↑

Arrows indicate a significant difference compared to adrenalectomised (ADX) mice receiving cholesterol replacement. No arrow indicates no significant difference. On days 1-9 mice received 15.0 mg/kg cocaine and on days 15 and 21 animals were given a 7.5 mg/kg cocaine challenge. * Enhanced response to the cocaine challenge when compared to saline-treated controls receiving similar substitution (data from **chapter 4**).

investigation. Piazza *et al.* demonstrated that administration of corticosterone 10 minutes prior to a session facilitates self-administration in LR rats [516]. In the present study, a slight degree of sensitisation was observed in mice receiving corticosterone 5 minutes prior to cocaine administration, although locomotor responses did not exceed those of ADX mice on day 9. In view of the findings of Piazza *et al.*, this suggests that there might be a non-genomic mode of action of the hormone, since the involvement of genomic effects that develop at 2 hours after corticosterone administration when the hormone concentrations are declining, is less likely. The rapid non-genomic effects of glucocorticoids can be mediated via both MR or GR putatively located in the cell membrane, that have considerably lower affinity for glucocorticoids than their 'classical' intracellular form [319,338,755]. Administration of MR or GR antagonists shortly prior to drug administration could be an interesting approach to investigate the involvement of non-genomic glucocorticoid actions. Furthermore, given the critical role for the ANS (**chapter 5**, see section 3.3 for discussion), it would be interesting to repeat the 2 hrs. and 5 mins. replacement studies in combination with epinephrine substitution.

An obvious line of reasoning to explain the insufficiency of corticosterone in fully reversing the ADX effect, would be to focus on experimental parameters such as the hormone dosage, the necessity of endocrine sensitisation and the possible requirement of ultradian and circadian rhythmicity. These aspects could very well play a role and are discussed in detail in **chapter 4**. However, the discrepancy in literature regarding the role of corticosterone in psychostimulant responsiveness, even when similar strains of animals are used, suggests that glucocorticoids are not the only factors of importance (e.g. [21,510]). Furthermore, two recent publications have proposed

that an elevation of glucocorticoids is necessary, but not sufficient, for the effects of stress on escalation of cocaine self-administration [417] and on morphine-induced conditioned place preference and dopamine release in the NAc shell [161]. For instance, in the study by Mantsch *et al.*, it was shown that stress-induced escalation of cocaine self-administration was prevented in ADX animals with diurnal corticosterone replacement. Administration of additional corticosterone (in the range of that induced by the stressor) by itself did not restore the ADX effect, while it was only effective in animals that were in addition exposed to the stressor [417]. This suggests that factors other than corticosterone may play an additional role in the effects of stress on the behavioural and reinforcing effects of psychostimulants.

In conclusion, corticosterone facilitates initiation and retention of sensitisation to the locomotor stimulant effects of cocaine in DBA/2 mice. However, a full restoration of the ADX effect was not achieved with various regimens of corticosterone replacement. Because stressors and psychostimulants activate the autonomic sympathetic nervous system (ANS) even more rapidly than the HPA-axis, studies were designed to investigate the actions of corticosterone in the context of epinephrine administration.

3.3 The role of the sympathetic nervous system

*In **chapter 5** it was demonstrated that the ANS and the HPA-axis act in a coordinate fashion to facilitate behavioural sensitisation of DBA/2 mice to cocaine.* Whereas neither corticosterone nor epinephrine alone were sufficient, both adrenal stress hormones were necessary to fully restore locomotor sensitisation of ADX mice to cocaine. Previous studies have demonstrated that the HPA-axis and the ANS are intimately linked during processes of learning and memory [53,571], however little attention has been dedicated to the role of the ANS in sensitivity to drugs of abuse.

The polar substance epinephrine does not cross the blood-brain-barrier. The catecholamine therefore most likely modulates brain function by activating β-adrenoreceptors on vagal afferents to the noradrenergic cell bodies in the solitary tract nucleus (NTS) [251,456,570,730]. Interestingly, cocaine itself increases availability of synaptic norepinephrine by blocking the NET [549]. The present data therefore suggest that additional noradrenergic stimulation (by peripheral epinephrine) may be required for full behavioural sensitisation in DBA/2 mice. Studies showing that adrenoreceptor agonists block stress-induced reinstatement of cocaine, heroin and alcohol seeking provide further evidence for the role of the noradrenergic system in mediating the effects of stress on drug responsiveness [193,376,620]. This would imply an important role for norepinephrine in psychostimulant sensitisation, a proposal

that is supported by most [182,183,587], but not all [691] studies. In the light of the present data (**chapter 2**), this discrepancy could be explained by the use of different strains and species. Furthermore, the considerable number of reports that glucocorticoids alone *are* sufficient to restore the effects of ADX, suggest that differences in sympathetic tone and, consequently, release of norepinephrine from sympathetic nerve terminals, may determine whether additional adrenergic stimulation is required.

The mechanism underlying the coordinate actions of corticosterone and epinephrine in cocaine sensitisation forms an interesting topic for further research. First, it would be of interest to investigate which of the hormones is rate-limiting. The observation that chronic corticosterone, but not repeated epinephrine administrations, partially reverses the sensitisation deficit in ADX mice (**chapters 4** and **5**), suggests that the glucocorticoid (if continuously present) is the primary regulator, whereas the role of epinephrine would be to facilitate the actions of corticosterone. However, this is highly speculative as these experiments involved 'artificial' conditions such as ADX, which by itself is likely to change the set point of the sympathetic nervous system, and hormone replacement in a non-physiological manner. Furthermore, especially during arousing tasks, there may be substantial norepinephrine release from sympathetic nerve terminals, which may have facilitated the actions of corticosterone. The independent contribution of adrenal glucocorticoids and epinephrine could be investigated by administration of MR and GR antagonists, or peripheral β-blockers.

Second, it is of great interest to investigate which brain regions are a substrate for the (inter)action of corticosterone and epinephrine, e.g. by microinjections of agonists and antagonists of glucocorticoid- (MR and GR) and adrenoreceptors. This may involve both direct [302,491] and indirect (via e.g. amygdala, hippocampus or PFC) [368,395,396,419,535,574] noradrenergic projections to the midbrain dopamine system. Of particular interest are the basolateral amygdala (BLA) and the NAc, as these brain regions are critical for the effects of glucocorticoids and epinephrine on memory consolidation [437,572-574,616].

Taken together, behavioural sensitisation of DBA/2 mice to cocaine depends on adrenal glucocorticoids. These hormones play a role during initiation, rather than expression, of sensitisation. Furthermore, the glucocorticoid action appears to require activation of the sympathetic nervous system. Regarding the time frame of action, a continuous presence of corticosterone is most effective in facilitating behavioural sensitisation, whereas the effects of the intermittent hormone substitutions (2 hrs. and 5 mins. prior to cocaine) need to be revisited in the light of the potential necessity of ANS activation.

4. PERSPECTIVES

Clinical and experimental studies point to a role for adverse life-events and glucocorticoid hormones in vulnerability to the reinforcing effects of drugs of abuse. This thesis revealed that susceptibility to the psychostimulant effects of cocaine results from complex interactions between genetic factors, the HPA-axis and the sympathetic nervous system. Our studies in mice of two genetic backgrounds show that certain individuals may acquire long-term neural and behavioural adaptations to cocaine independent of stress hormones, whereas in others adrenal glucocorticoids *and* catecholamines constitute important risk factors.

Figure 1 depicts the interplay between adrenal corticosteroids and catecholamines in the process of behavioural sensitisation to cocaine. In agreement with observations in other laboratories, it was demonstrated that glucocorticoids facilitate initiation of sensitisation [240,418,514,516], but only in certain genotypes (the DBA/2 strain). However, the present studies point to an additional role for the sympathetic nervous system. Adrenal glucocorticoids and catecholamines act in a coordinate fashion to enhance behavioural sensitisation to cocaine, which may involve facilitated dopaminergic transmission in the nigrostriatal circuit and regulation of D2 receptor densities in the projection areas of the dopamine system. Furthermore, whereas glucocorticoids have a permissive role in adrenal epinephrine synthesis [583,738], the current data suggest that epinephrine in turn facilitates HPA-axis activation by cocaine at the level of the PVN. Therefore, under conditions of repeated cocaine exposure, the two adrenal hormones may mutually facilitate each other's synthesis and release in a feed-forward fashion, which could further enhance the contribution of these hormones to psychomotor sensitisation.

The present data open up a window of opportunities for further research.

First, the DBA/2 strain provides an interesting animal model to investigate the neural, endocrine and genetic mechanisms underlying vulnerability to psychostimulant drugs that may precipitate in response to adverse events. This inbred strain is susceptible to the influence of adrenal hormones and environmental manipulations and is characterised by considerable inter-individual variability in cocaine responsiveness (**chapter 2**). Identification of population extremes, so-called 'responders' and 'non-responders', and subsequent phenotyping constitutes one approach to investigate precipitation of drug responsiveness by stress hormones. Alternatively, manipulations in early life (e.g. lack of maternal care) or in adulthood (e.g. exposure to various chronic stressors) can be applied to further discriminate phenotypes that are relatively resistant or hypersensitive to psychostimulants. Combining these

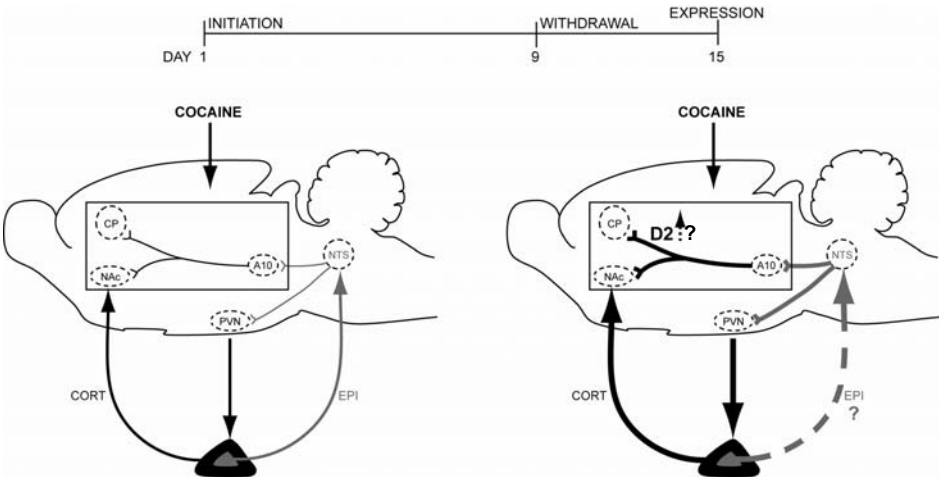

Figure 1: *Proposed model for the interaction between adrenal glucocorticoids and epinephrine in behavioural sensitisation to cocaine.*
The model represents the DBA/2 strain that is 'susceptible' to the impact of adrenal hormones on initiation of behavioural sensitisation to cocaine. Corticosterone (cort) and epinephrine (epi) act or interact in a coordinate fashion to facilitate initiation of sensitisation via adaptations in the nigrostriatal dopamine system and the NAc core that may include i) greater reactivity of nigrostriatal neurons, and ii) alterations in D2 receptor signalling in the terminal fields, possibly towards supersensitivity as reported elsewhere [306,675,695]. Corticosterone can enter the reward-related brain areas directly, whereas epinephrine activates the noradrenergic system in the nucleus of the solitary tract (NTS). With repeated drug exposure, there is enhanced secretion of corticosterone, which may be further facilitated by epinephrine, and possibly also increased epinephrine release from the adrenals. The resulting feed-forward cascade involving epinephrine and corticosterone interactions may further facilitate behavioural sensitisation to cocaine. PVN: hypothalamic paraventricular nucleus, NAc: nucleus accumbens, CP: caudate putamen.

approaches with genome-wide microarray gene expression profiling on reward-related brain regions may lead to the identification of new pathways and molecular targets that underlie susceptibility to psychostimulant drugs and dopaminergic psychoses in general.

Second, an important next step involves the extrapolation of the present results to other models for drug addiction in laboratory animals, such as the self-administration paradigm. It would be of great interest to investigate the coordinate action of corticosteroids and catecholamines in acquisition, maintenance and relapse of self-administration. The present data, which show a critical role for adrenal hormones in the initiation of behavioural sensitisation, suggest that these hormones play a role in acquisition of drug self-administration. This has indeed been demonstrated

for corticosteroids [418,516], however the role of epinephrine is unknown. Furthermore, given that glucocorticoid hormones act in a context-dependent fashion, it would be interesting to investigate the role of adrenal hormones in cue-controlled cocaine seeking and relapse, e.g. under a second-order schedule of reinforcement.

Third, the mechanism and brain circuitry via which the ANS and the HPA-axis modulate psychostimulant responsiveness form an important focus for further research. With respect to the ANS, this issue can be addressed by local or systemic administration of α- and β-adrenoreceptor agonists and antagonists prior to an experimenter- or self-administered drug infusion. This could be combined with telemetry or continuous blood sampling to monitor the activity of the ANS. Agonists/antagonists that are incapable of crossing the blood-brain-barrier would provide a useful tool to distinguish between the central and peripheral actions of catecholamines. Glucocorticoid actions can be manipulated systemically or centrally via the 'classical' endocrine and pharmacological methods as applied in this thesis, as well as by the use of transgenic animals or the application of small-interference (si)RNA-mediated knock-down of GR and MR (and their targets) in distinct brain regions. The initial focus could be on the dopaminergic and noradrenergic nuclei, the primary substrates for cocaine and epinephrine in the brain, thereafter being extended to brain regions such as the amygdala, prefrontal cortex and hippocampus that provide the midbrain dopamine system with excitatory or inhibitory input.

Fourth, whereas there is a wealth of data on the influence of stress and glucocorticoids on psychostimulant sensitivity, an interesting line of further research would be to study the role of adrenal hormones in the behavioural and reinforcing effects of different classes of drugs of abuse, such as opioids, nicotine, ethanol and cannabinoids. Indeed, there is limited evidence that corticosteroids differentially facilitate dopamine-mediated behavioural responses to cocaine and morphine [422]. Furthermore, whereas direct or indirect activation of the midbrain dopamine system is a commonality shared by all drugs of abuse, distinct classes of drugs have diverging pharmacological actions that may be differentially influenced by adrenal hormones.

Finally, the implications of the present findings for human drug addiction remain to be investigated. In individuals with cocaine dependence, acute cortisol can trigger craving [192] and plasma cortisol concentrations are positively correlated with the subjective effects of the drug and drug-induced dopamine release in the midbrain [486]. Despite the controversy regarding the role of glucocorticoids in established drug responsiveness (the present data, [153,168]), this suggests that GR antagonists may have therapeutic potential in reducing some of the subjective and neurochemical effects of cocaine. Interestingly, recent clinical trials have demonstrated that the GR antagonist mifepristone may have efficacy in the treatment of psychotic major depression

Box 2: *Stereotypy.*

In the present studies, locomotion was measured as an index of behavioural responsiveness to cocaine. However, the effects of psychostimulants in rodents are dose-dependent and can initiate a spectrum of behavioural responses. Whereas lower doses induce horizontal locomotor hyperactivity, higher doses produce stereotyped behaviours; repetitions of movements that are part of the normal behavioural repertoire including head shaking, rearing, sniffing, gnawing, licking and circling. In humans, stereotyped behaviours are symptoms of a number of psychiatric disorders [262,640,667]. Stereotypy is an issue that deserves attention in the light of gene x environment interaction in psychostimulant responsiveness.

It has been proposed that stereotypy serves a coping function that reduces drug-induced arousal and stress [410,454]. Indeed, an amphetamine sensitisation regimen that significantly increases stereotyped behaviours, has been shown to attenuate the normal amphetamine-induced elevation in corticosterone [454]. Conversely, a decrease in amphetamine stereotypy induced by mesostriatal dopamine depletion prolonged the amphetamine-induced elevation in corticosterone secretion [322]. Furthermore, stressful conditions can enhance amphetamine- and cocaine-induced stereotypy, and this has been proposed to occur only when the stress is uncontrollable [410]. Thus, coping is an important variable in the interaction between the HPA-axis and psychostimulant drugs, which may be reflected by stereotyped behaviour [410].

Strain differences may exist in the susceptibility of laboratory rodents to psychostimulant-induced stereotypies. This has also been demonstrated for the C57BL/6 and DBA/2 strains, the latter but not the former, developing stereotyped behaviours during the course of repeated cocaine (32 mg/kg) treatment [666] or in response to food restriction [67]. In the present studies, the test setting did not allow for the detailed behavioural analysis required to distinguish subtle stereotyped movements. It is therefore conceivable that a difference in stereotyped responding may have contributed to the observed strain differences in behavioural sensitisation to cocaine. However, the doses of cocaine used in the present study (15.0 mg/kg during the treatment phase and 7.5 mg/kg for the drug challenge) were relatively low and, especially the challenge dose is not likely to have induced a high degree of stereotypy [45]. Furthermore, the observation that corticosterone secretion was attenuated by cocaine in the C57BL/6 strain that displayed high horizontal locomotion throughout the sensitisation paradigm (**chapter 2**), contradicts the notion that stereotypy might function to attenuate HPA-axis responsiveness in this strain.

The possibility of stereotypy in ADX animals should also be considered. Activation of the HPA-axis and the ANS enable an organism to respond and adapt to stressful situations and are therefore part of their coping mechanism. ADX mice are however not capable of releasing adrenal corticosterone or catecholamines in response to any stimulus. It is therefore conceivable that ADX animals develop stereotyped behaviours to cope with cocaine-induced arousal. In this line of reasoning, stereotypy would be part of the coping response not only in animals that are exposed to too high, but also too low levels of stress hormones. Further studies are therefore required to investigate whether stereotypy plays a significant role in the behavioural response of ADX mice to cocaine, especially given the seemingly high susceptibility of DBA/2 mice to develop such behavioural responses.

[34,160]. Moreover, mifepristone selectively reduced psychotic rather than depressive symptoms, which suggests that the antagonist modulates dopaminergic transmission [160]. Therefore, it would be interesting to test the effects of this compound on drug craving in human cocaine addicts. Clearly, more research is required to investigate the involvement of the sympathetic nervous system alone, and in interaction with various components of the HPA-axis, in human drug addiction. The present data suggest that β-blockers may have therapeutic potential. One first approach could be to investigate whether, in addition to cortisol secretion, the activity of the ANS (e.g. heart frequency) is a parameter that correlates with the extent of drug craving, and whether craving can be reduced by administration of β-blockers.

The data presented in this thesis indicate that, depending on genetic background, stress hormones can provide important risk factors for vulnerability to the psychostimulant effects of cocaine. Furthermore, these data add a new dimension to the concept of stress in addiction research, extending the focus from being solely on glucocorticoids to a wider scope involving the HPA-axis and the sympathoadrenal system with their central targets.

5. CONCLUSIONS

i) *Genetic background* determines sensitivity to the psychostimulant effects of cocaine and the contribution of *adrenal hormones*. One strain was identified, the DBA/2 strain, that is susceptible to the influence of adrenal stress hormones on behavioural sensitisation to cocaine.

ii) The interaction between *genetic background* and *adrenal stress hormones* in cocaine sensitivity can be monitored by measuring markers in the midbrain dopamine system. Most notably the D2 receptor in the terminal fields of the nigrostriatal dopamine system and the NAc core is an interesting candidate to mediate this interaction.

iii) The initiation phase of behavioural sensitisation is a critical *time-window* for the action of adrenal glucocorticoids. During this phase, continuous presence of high glucocorticoid concentrations facilitates sensitisation, whereas it remains to be established whether this involves non-genomic and/or genomic mechanisms.

iv) The sympathetic nervous system may signal aspects of the *physiological context* in which glucocorticoids operate. Adrenal epinephrine and corticosterone act in a coordinate fashion to facilitate behavioural sensitisation to cocaine.